Chemistry & Biology
Preview

Interactome of ErbB4 Unveiled

Terry T. Takahashi[1] and Shuwei Li[2,*]
[1]Department of Chemistry, University of Southern California, 925 Bloom Walk, HED 216, Los Angeles, CA 90089, USA
[2]Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, 9600 Gudelsky Drive, Rockville, MD 20850, USA
*Correspondence: liw@umbi.umd.edu
DOI 10.1016/j.chembiol.2008.08.001

MacBeath and colleagues (Kaushansky et al., 2008) use a protein array technology to find binding partners of ErbB4 in a genome-wide and quantitative fashion, shedding new light on how ErbB4 initiates cellular signaling events and why ErbB4 is not a potent oncogene.

The ErbB protein family, also named the epidermal growth factor receptors (EGFR) family, is a subfamily of receptor tyrosine kinases (RTKs) with four structurally related members: EGFR, ErbB2, ErbB3, and ErbB4. These receptors play critical roles in a broad array of biological processes, such as cell proliferation and differentiation, and are essential for normal human development (Citri and Yarden, 2006). For instance, insufficient ErbB signaling is often associated with neurodegenerative diseases, while overexpression or constitutively active mutants of ErbB receptors are commonly observed in various cancers, suggesting that their activity in vivo must be tightly regulated. Therefore, it is of great interest to understand the functions that ErbB receptors play in cellular signaling networks. However, although the first three members of this protein family are well studied, little is known about ErbB4, whose functions in vivo remain elusive.

In this issue of *Chemistry & Biology*, MacBeath and colleagues utilize a quantitative, non-biased, and high-throughput method to elucidate the functions of ErbB4 by determining the affinities and identities of its downstream interaction partners (Kaushansky et al., 2008). ErbB4 and other members of the ErbB family share the same structural topology, with a ligand-binding extracellular region, a single transmembrane domain, a cytoplasmic kinase catalytic domain, and a C-terminal tail containing a number of tyrosine residues. Interestingly, while EGFR and ErbB4 are fully functional receptors for signaling, ErbB2 and ErbB3 are defective because ErbB2 has no soluble ligands and the kinase domain of ErbB3 is inactive. Therefore, ErbB4 is thought to initiate signaling by adopting the same mechanism as EGFR, in which ligand-induced homo- or heterodimerization of the extracellular domains brings two neighboring kinase domains together, resulting in an asymmetric conformational change and enabling cross-phosphorylation of the C-terminal tails (Zhang et al., 2006). This activation provides docking sites to recruit downstream enzymes and adaptor proteins that contain either SH2 (Src homology 2) or PTB (phosphotyrosine-binding) domains, which specifically recognize both phosphotyrosine (pTyr) and its adjacent residues. Indeed, a recent structural study of ErbB4 activation confirms this asymmetric mechanism is well reserved among ErbB family members (Qiu et al., 2008). Furthermore, similar to EGFR, ErbB4 can also form heterodimers with ErbB2 and ErbB3. From all aspects, ErbB4 should just be another potent oncogene as other ErbB receptors are. This is not the case, however, as ErbB4 can also act as a tumor suppressor (Gallo et al., 2006). Why does ErbB4 have such a remarkable capability? The answer to this question may rely on the identification of what signaling pathways can be trigged by ErbB4. As the first step, it will be useful to identify SH2/PTB-containing proteins that interact with ErbB4.

In order to study phosphorylation-dependent protein-protein interactions, synthetic pTyr-containing peptides are used as surrogates of normally difficult to prepare full-length phosphorylated proteins. Phosphorylated peptides can act as substitutes for the full-length proteins since SH2 and PTB domains normally bind and recognize only a few amino acids that flank a specific pTyr. However, because ErbB4 contains multiple potential pTyr sites and there are a large number of SH2 (>100) and PTB (>40) domains in human genome, it is daunting and impractical to test each potential binding pair by low-throughput biochemical analysis.

Fortunately, several high-throughput approaches have already been developed. Bidlingmaier and Liu selected proteins interacting with the autophosphorylation site (Y1173) of EGFR from a yeast surface-displayed human cDNA library by iteratively enriching clones that bind to the corresponding pTyr peptide, which should be applicable on the study of ErbB4 as well (Bidlingmaier and Liu, 2006). Schulze et al. used a pair of phosphorylated and nonphosphorylated peptides to pull down interacting proteins from cell lysates that were isotope-labeled (Schulze et al., 2005). This permitted a quantitative analysis with mass spectrometry (MS) to distinguish phosphorylation-specific interaction partners from nonspecific background. A few binding proteins for each ErbB receptor were identified in this way. Neither method, however, can provide a complete and quantitative measurement of the ErbB receptors with all SH2/PTB domains.

To overcome this challenge, MacBeath and colleagues constructed a protein array featuring almost every SH2 domain and PTB domain in human genome (Jones et al., 2006). They then performed a thorough literature survey to find all phosphorylation sites on ErbB receptors and synthesized the corresponding fluorescently labeled pTyr-containing peptides. In the case of ErbB4, in which no experimentally verified pTyr sites were reported, four peptides with predicted phosphorylation sites were prepared.

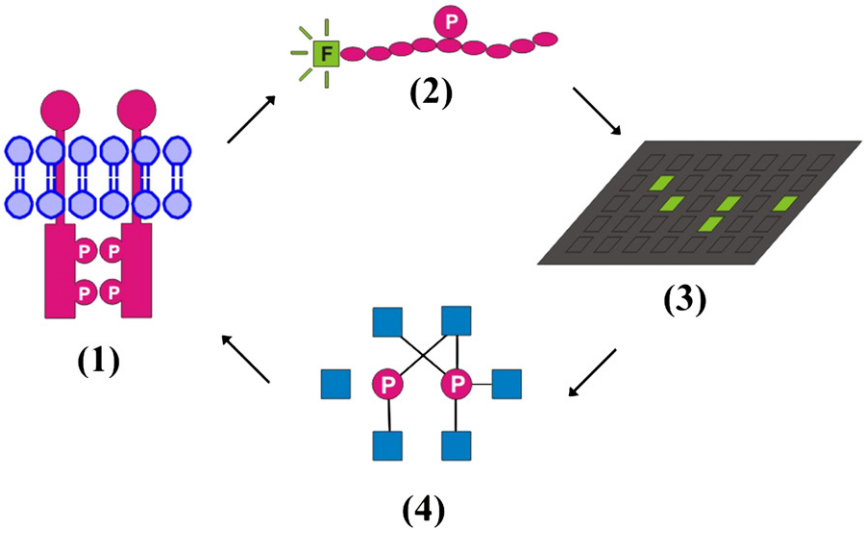

Figure 1. Flowchart of Quantitative Mapping of ErbB Receptors Interactome
(1) Identification of pTyr sites by literature search or MS assay.
(2) Synthesis of fluorescently labeled pTyr-containing peptides.
(3) Detection of interaction with protein arrays.
(4) Bioinformatics analysis.

These peptides were used to probe the protein array in a series of concentration-dependent assays to determine the binding affinity between each pTyr-containing peptide and each protein on the array (Figure 1). In addition to confirming known interactions that had been reported previously, more than 100 previously unrecognized interactions were identified quantitatively by this high-throughput platform, which are difficult to acquire by other methods.

Continuing their efforts to understand the paradoxical functions of ErbB4, MacBeath and colleagues first identified 19 pTyr sites on ErbB4 by using tandem MS, then applied their high-throughput protein array analysis with these newly identified pTyr-containing peptides (Kaushansky et al., 2008). They identified known interaction partners of ErbB4 as well as a number of previously unknown partners, one of which—signal transducer and activator of transcription 1 (STAT1)—was confirmed to interact with ErbB4 by further biochemical experiments.

This high-throughput approach to explore the entire interactome of ErbB4 receptor is a technical triumph, illustrating that protein arrays offer an unparalleled capability to simultaneously determine the affinity between ErbB4 and every SH2/PTB domain in human genome. In addition, the quantitative nature of this measurement makes it possible to draw a picture of the interaction network at any defined affinity threshold, providing new insights into the signaling properties of ErbB4 at different expression levels. More importantly, this unbiased and streamlined method is generally applicable for the study of any receptor or nonreceptor tyrosine kinase, representing a quantum leap toward the characterization of all signaling networks regulated by tyrosine phosphorylation. Finally, these experiments provide a comprehensive view of ErbB4 functions.

Surprisingly, ErbB4 is recognized by fewer SH2 and PTB domains than EGFR, ErbB2, and ErbB3, implying ErbB4 activates fewer pathways than other EGF family receptors. Therefore, its unusual property to suppress tumor growth may not result from its ability to initiate some unknown signaling circuits, but may instead result from its higher selectivity in recruiting downstream partners. Since ErbB4 can form heterodimers with other ErbB receptors, ErbB4 may exert a negative dominant effect by forming more benign heterodimers with itself, thereby reducing the level of more oncogenic ErbB dimers. It is tempting, therefore, to hypothesize that activation of a greater number of signaling pathways increases the likelihood of oncogenic transformation, a hypothesis that will require much more testing, but whose answers are within the reach of systems biology.

REFERENCES

Bidlingmaier, S., and Liu, B. (2006). Mol. Cell. Proteomics *5*, 533–540.

Citri, A., and Yarden, Y. (2006). Nat. Rev. Mol. Cell Biol. *7*, 505–516.

Gallo, R.M., Bryant, I., Fry, R., Williams, E.E., and Riese, D.J., 2nd. (2006). Biochem. Biophys. Res. Commun. *349*, 372–382.

Jones, R.B., Gordus, A., Krall, J.A., and MacBeath, G. (2006). Nature *439*, 168–174.

Kaushansky, A., Gordus, A., Budnik, B.A., Lane, W.S., Rush, J., and MacBeath, G. (2008). Chem. Biol. *15*, this issue, 808–817.

Qiu, C., Tarrant, M.K., Choi, S.H., Sathyamurthy, A., Bose, R., Banjade, S., Pal, A., Bornmann, W.G., Lemmon, M.A., Cole, P.A., et al. (2008). Structure *16*, 460–467.

Schulze, W.X., Deng, L., and Mann, M. (2005). Mol. Syst. Biol. *1*, 2005.0008.

Zhang, X., Gureasko, J., Shen, K., Cole, P.A., and Kuriyan, J. (2006). Cell *125*, 1137–1149.

